

Breast and Prostate Cancer: Basic Mechanisms

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100 CHROMOSOME 16q ALLELIC LOSSES AT PREINVASIVE STAGES OF BREAST CANCER.
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In allelotypic studies from microdissected paraffin sections we have recently observed that involvement of chromosome arms 1p, 3p, 3q, 6p, 16p, 18p, 18q, 22q, and possibly 6q and 11p appear to be late events in breast cancer progression since allelic losses or imbalances affecting these chromosome arms were observed with very low frequency at the *in situ* stage. On the other hand allelic imbalances and losses affecting chromosome arms 7p, 16q, 17p and 17q appear to be early abnormalities since they were observed in a significant number of Ductal Carcinoma *in situ* lesions. We are currently performing a deletion mapping analysis of chromosome 16q at preinvasive stages of breast cancer. We observed that the region between markers D16S898 and D16S893 (16q 22.1-24.1) appears as the most frequently deleted area. Adjacent tissue sections of the same tumors are also being analyzed by fluorescence *in situ* hybridization of chromosome 16. These studies will allow to better define the target and understand the chromosomal mechanisms leading to the 16q allelic losses at preinvasive stages of breast cancer. Supported by U.S. Army DAMD17-94-J-4078.

101 DEVELOPMENT OF TISSUE CULTURE MEDIA SELECTIVE FOR THE GROWTH OF BOTH NORMAL & MALIGNANT HUMAN BREAST EPITHELIAL CELLS.

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The invasive cells in primary breast carcinomas show a keratin phenotype that is characteristic of the luminal epithelial cell. However, primary breast cancer biopsies consist of a mixture of different cell types which often include normal as well as malignant epithelial cells. As yet there is no standard procedure that allows routine culturing of demonstrably malignant cancer cells to respond to conventional culture media and also because such cultures are often overgrown by normal cells of both stromal and epithelial phenotypes. The two predominant epithelial cell types, luminal and myoepithelial, can be distinguished on the basis of the pattern of keratins they express. It is the myoepithelially-derived cells that proliferate more rapidly *in vitro* (O'Hare et al., 1991) and often tend to overgrow primary tumour cultures. Ideally, one would wish to use a selective medium that minimised such overgrowth whilst retaining viability of the malignant epithelial cells. Thus we have used a novel immunomagnetic cell separation system (MACS) (Clarke et al., 1994) to examine systematically the different media requirements for sorted populations of luminal epithelial and myoepithelial cells isolated from reduction mammaplasty patients. Different media have been identified in which either luminal epithelial or myoepithelial growth is specifically favoured. These systems now provide the opportunity to study the specific growth requirements of the malignant cells with the aim of developing culture conditions selective for human breast cancer cells. This would facilitate the application of techniques for identifying molecular abnormalities associated with the early stages of malignancy.

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102 DD3: A NEW PROSTATE SPECIFIC MARKER, OVEREXPRESSED IN PROSTATIC TUMORS.

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In order to identify genes differentially expressed during prostate cancer development, we applied the technique of differential display analysis using mRNA from normal, benign hyperplastic and tumor prostatic tissue from the same patients. We thus identified DD3, which on Northern blot detects two transcripts that are specifically expressed in human prostatic tumors whereas no expression of these transcripts is found in normal or BPH tissue. Nucleotide sequence analysis of DD3 did not reveal an open reading frame nor did we find homology with any known gene. Isolation of additional DD3-related cDNA and genomic clones allowed a further characterization of the transcription unit of DD3 and revealed that alternative splicing occurs, which may be the mechanism giving rise to the differently sized transcripts. Using the DD3-related genomic clones as probes, we were able to map DD3 to chromosome 9q21-22, a region which (by CGH) was shown to be amplified in a number of prostatic tumors, suggesting that overexpression of the gene may be a result of gene amplification. Upon developing primers for RT-PCR, we were able to show that DD3 expression is very prostate specific since no DD3 transcripts could be detected in normal human artery, bladder, colon, duodenum, heart, kidney, liver, lung, pancreas, skin or spleen. Also in the human prostate cancer cell lines LNCaP, Du145, PC3 and TSU and in a number of human kidney cancer cell lines no DD3-related PCR product could be amplified. We are currently establishing whether we can use RT-PCR analysis of DD3 to detect prostate cancer cells in the peripheral blood of patients with metastatic disease. Furthermore, we will try to gain insight in the function of DD3 and its role in prostate cancer development.

103 IDENTIFICATION OF PROTEINS WHICH INTERACT WITH BRCA-1.

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The search for specific genes involved in breast cancer development has led to identification and isolation of BRCA-1, a gene where mutation is associated with a high lifetime risk from breast or ovarian cancer. Although genetic studies suggest a strong correlation between BRCA-1 mutations and cancer development, the specific contribution of such mutations to this process is not known. Moreover, there is little evidence indicating a causal role for BRCA-1 in spontaneous cancer. Accordingly, our work has focused on identifying proteins which interact with BRCA-1 as an approach to facilitate understanding of its role in normal growth or cancer progression.
Using baculoviral-based vectors, we have expressed several segments of BRCA-1 as GST fusion proteins in Sf-9 cells. These fusion proteins were used as affinity reagents to capture potential BRCA-1 associating proteins from radiolabeled breast cell lines. We have identified at least 2 proteins from MCF-7 cells which appear to associate with different regions of BRCA-1. The first protein, which interacts with a C-terminal domain, migrates on SDS-PAGE with an apparent molecular weight of ~ 30 kDa. The other protein has an apparent molecular weight of ~ 200 kDa and associates with a region of BRCA-1 encoding part of exon 11. We are currently determining whether either of these proteins exhibit differential binding to wild-type and mutant forms of BRCA-1. In addition, we are working to obtain preliminary sequence information for these proteins.